

EXHIBIT D

TITLE Specificity studies w/ Chlamydia probes

From Page No. 34

Prepare hybridization mixes

lysates:

30 μ l DIBSS diluent
10 μ l lysate
360 μ l DIBSS 1 probe
400 μ l

Do duplicates

Pos. Control

30 μ l DIBSS

1 μ l Chlamydia RNA or 10 μ l E. coli RNA
+ 9 μ l H₂O
300 μ l DIBSS 1 probe
400 μ l

Incubate 1 hr 64°C

Neg. control

30 μ l DIBSS
10 μ l 3.2% SDS
360 μ l DIBSS 1 probe
400 μ l

Add 4.5 ml Separation solution .14M PB LN 60278

Incubate 5' at 64°C

Centrifuge

Decant SN into 30 ml scintillation vial

Add 5.0 ml Wash solution, vortex LN 60276 .14M PB

Incubate 5' at 64°C

Centrifuge

Decant SN into same scintillation vial as 1st wash

1. Count HA pellets and wash fraction for 32 P counts

2. Add 5.0 ml eyekart to HA pellet

Add 15.0 ml p gel to wash vial

Count again α jet 32 P counts

Used Sal for counting 2x2'

Prepare blanks ① spin down 4.5 ml HA and count

② add 9.0 ml .14M wash and to scint.

Recorded by

To Page No. 36

Witnessed & Understood by me,

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TITLE

From Page No. 38

Purpose: To assay lysates of Chlamydia strains for cross reactivity with probes at 60°C and 176 at 64°C.

Reagents: Same as for previous experiment (405:34)

In addition used lysates for C. trachomatis 300, 301, 302, 303 (other serotypes than the one used to make the probes and found in humans)

From Mary NB 435:3

Diluted 7H RNA 25 µg/ml (334:10) 1.5 to .05 µg/ml
Make up probe solutions

① To 8.4 ml D/PSS mix, add 5 µl ³H-probe
NB 391:25 mixed and 20 µl I
Probe 1082 (22,000 cpm/ml) iodinated by
Kevin NB 388:42

②

To Page No. 40

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TITLE Specificity Studies with Chlamydia probes

From Page No.

Assay Protocol: Prepare hybridization mixtures in scintillation vials.

Lysates:	30 μ l DIBSS diluent	W.C.:	30 μ l DIBSS diluent
10 μ l lysate		10 μ l 3.2% SDS	
360 μ l DIBSS/probe mix		860 μ l DIBSS/probe mix	
400 μ l total volume		400	

711 RNA:	30 μ l DIBSS diluent	
2 μ l RNA 0.5 μ g/ml		
8 μ l H ₂ O		
360 μ l DIBSS/probe		
400		

E. coli RNA:	30 μ l DIBSS diluent	
10 μ l RNA 1.0 μ g/ml		
360 μ l DIBSS/probe		
400		

Incubate ChA 176 probe rxn at 64°C 1 hr.

Separate hybrids and wash ^{AB 405} as p. 35Count in HAL 2x2' on
10 μ l ³²P counts Program 1

Add 0.09 ml of probe mixture to HA pellet in scint vial
 Add 5.0 ml cytosine and count use this value
 for 10 ml counts for ³⁵I-hybridization w/ pan bacterial pool
 Add 5.0 ml cytosine, and count in HAL 2x2'
 10 μ l ³⁵I counts. ^{AB 405:34}
 (Did not count washes for ³⁵I as did in previous exp.)
 E. coli RNA sample 2 was spilled for probe ChA 176.

Results: see p. 41

Conclusions: Probes 176 and reacted with all C. trachomatis strains but not with the C. psittaci. The ³⁵I ^{AB} 4082 data showed that RNA was present in all tubes.

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Witnessed & Understood by me,

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TITLE Specificity Study of Chlamydia probes

Proj ct N 3

B k N 405

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From Page No. 40

Cat A 126		340			
	SMPL	CPM	TOTAL	%	
711	1 FP	929.8	2091.0	44.5	
	BP	1161.3		55.5	
801	2 FP	942.3	2166.0	43.5	
	BP	1223.8		56.5	
802	3 FP	623.0	2210.0	28.2	
	BP	1587.0		71.8	
803	4 FP	631.8	2179.3	29.0	
	BP	1547.5		71.0	
804	5 FP	561.5	2180.3	25.8	
	BP	1618.8		74.2	
805	6 FP	536.8	2159.5	24.9	
	BP	1622.8		75.1	
806	7 FP	566.5	2147.5	26.4	
	BP	1581.0		73.6	
807	8 FP	610.8	2248.3	27.2	
	BP	1637.5		72.8	
808	9 FP	551.3	2178.0	25.3	
	BP	1626.8		74.7	
809	10 FP	563.5	2124.0	26.5	
	BP	1560.5		73.5	
810	23 FP	2278.5	2295.0	99.7	
	SP	16.5			
811	12 FP	2313.5	2328.0	99.4	
	BP	14.5		.6	
812	13 FP	2228.5	2239.3	99.5	
	BP	10.8		.5	
813	14 FP	2284.0	2295.5	99.5	
	BP	11.5		.5	
814	15 FP	2303.3	2319.5	99.3	
	BP	16.3		.7	
815	16 FP	2302.5	2318.3	99.3	
	BP	15.8		.7	
816	17 FP	403.0	2152.0	18.7	
	BP	1749.0		81.3	
817	18 FP	310.0	2073.8	14.9	
	BP	1763.8		85.1	
818	19 FP	2202.8	2217.3	99.3	
	BP	14.5		.7	
819	20 FP	598.8	597.3	98.9	
	BP	6.5		1.1	
820	21 FP	2290.5	2316.3	98.9	
	BP	25.8		1.1	
821	22 FP	2255.3	2278.0	99.0	
	BP	22.8		1.0	

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Witnessed & Understood by me,

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TITLE Specificity Studies with Eco A ~~23#~~

From Page No. _____

Procedure: Results:

ECOR 1082 with 176

TOTAL 1: 13760

SAMPLE	CPM	H% %	AVG% %	XBKGD
711	6849	49.8		
	7170	52.1	50.9	
300	8420	61.2		
	8571	62.3	61.7	
301	9490	69.0		
	9610	69.8	69.4	
302	8809	64.0		
	9557	69.5	66.7	
303	9153	66.5		
	8793	63.9	65.2	
751	7520	54.7		
	7666	55.7	55.2	
764	7692	55.9		
	7477	54.3	55.1	
768	4677	34.0		
	4483	32.6	33.3	
RNA, 711	8877	64.5		
	9516	69.2	66.8	
coli.	8431	61.3	61.3	
N.C.	139	1.0		
	132	1.0	1.0	

% Hybridization³²P-Eco A 176³²P-Eco A 1082Lysates

C. trachomatis	711	56.0	50.9
"	300	71.4	61.7
"	301	74.5	69.4
"	302	73.2	66.7
"	303	74.1	65.2
C. psittaci	751	0.7	55.2
"	767	0.5	55.1
"	768	0.7	33.3

RNA

C. trachomatis	711	83.2	66.0
E. coli		0.9	61.3
N.C.		1.1	1.0

Hybridization
Assays done at 60°C

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